Tomato chlorotic mottle Guyane virus: a novel tomato-infecting bipartite begomovirus from French Guiana

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Tomato chlorotic mottle Guyane virus: a novel tomato-infecting bipartite begomovirus from French Guiana

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Abstract This is the first description of the complete genome sequence of a new bipartite begomovirus isolated from tomato (*Solanum lycopersicum*) in French Guiana, for which we propose the tentative name "tomato chlorotic mottle Guyane virus" (ToCMoGFV). DNA-A and -B nucleotide sequences of ToCMoGFV are only distantly related to known New World begomoviruses. They share the highest nucleotide sequence identity of 80 % with the Brazilian isolates of macroptilium yellow spot virus (MacYSV) and 73 % with soybean chlorotic spot virus (SBCSV). Phylogenetic analysis demonstrated that this novel virus belongs to a new lineage of New World bipartite begomoviruses. The discovery of this new virus confirms the high genetic diversity of begomoviruses in Latin America.

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Begomoviruses (genus *Begomovirus*, family *Geminiviridae*) are a group of plant viruses that are transmitted by the whitefly *Bemisia tabaci* (Aleyrodidae) and are responsible for serious diseases in a wide range of cultivated crops, including tomato (*Solanum lycopersicum*) (for review, see ref. [13]). In the last two decades, a diverse group of indigenous begomoviruses, commonly known as New World (NW) begomoviruses, has emerged as a major threat to tomato production in Latin America [1, 5, 6, 11, 12]. Except for the recently described indigenous monopartite virus tomato leaf deformation virus (ToLDeV) in Ecuador and Peru [9], all indigenous NW begomoviruses have a bipartite genome (two DNA components, each approximately 2.6 kb, referred to as DNA-A and DNA-B). Since the first reports of tomato diseases probably associated with begomoviruses in Brazil in the 1960s [1, 5], a complex of more than 20 begomoviruses has been described on tomato in Latin America and the Caribbean [3].

In July 2010, leaf samples from tomato plants showing severe symptoms of leaf curling and yellowing resembling tomato yellow leaf curl disease (TYLCD) were collected in French Guiana (Table 1) and preserved by dehydration using anhydrous calcium chloride. Total DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN). Viral genomes were amplified by rolling-circle amplification using *Phi29* DNA polymerase [7]. Amplified products were digested with *Xba*I and *Nco*I endonucleases. The monomeric full-length DNA-A and DNA-B molecules obtained (~2.6 kb) were purified and ligated into the pGEM-3Zf vector (Promega). The ligated products were then cloned into *Escherichia coli* (JM 109). The isolated genomes were completely sequenced by primer walking (Macrogen), and contigs were assembled with DNA Baser v.2.91 (Heracle BioSoft). First, DNA-A and DNA-B nucleotide sequences were subjected to a BLAST search for preliminary species

Table 1 Geographical origin of tomato samples and description of French Guiana isolates of tomato chlorotic mottle Guyane virus (ToCMoGFV)

Date of sampling (dd/mm/yyyy)	Locality[#]	GPS coordinates		Isolate acronym	DNA-A GenBank accession no.	DNA-B GenBank accession no.
		Latitude	Longitude			
06-07-2009	Stoupan	4.728303	-52.324996	[GF:Sto1:GF435:09]	KR263179	KR263178
06-07-2009	Stoupan	4.728303	-52.324996	[GF:Sto2:GF462:09]	-	KR263175
06-07-2009	Stoupan	4.728303	-52.324996	[GF:Sto3:GF453:09]	-	KR263176
06-07-2009	Montsinéry	4.891269	-52.493338	[GF:Mon1:GF451:09]	KR263180	KR263174
06-07-2009	Montsinéry	4.891269	-52.493338	[GF:Mon2:GF455:09]	KR263181	KR263172
06-07-2009	Montsinéry	4.891269	-52.493338	[GF:Mon3:GF436:09]	-	KR263173
08-07-2009	Kourou	5.154824	-52.645413	[GF:Kou:GF454:09]	-	KR263177

[#] Each sample corresponds to a distinct leaf from a different plant

assignment. Multiple sequences were aligned by the MUSCLE alignment method, and maximum-likelihood (ML) phylogenetic trees were constructed by the PHYML method implemented in MEGA v.6.06 [14]. Pairwise identity comparisons of nucleotide sequences were performed with pairwise deletion of gaps using SDT v1.2 [10].

Three DNA-A and seven DNA-B nucleotide sequences were obtained, with features typical of New World bipartite begomoviruses (Table 1). DNA-A components, 2631/2633 nucleotides in length, shared more than 99.8 % nucleotide sequence identity. They had five open reading frames (ORFs): AV1 (249 aa; capsid protein [CP]) in the viral sense, and AC1 (344 aa; replication-associated protein [REP]), AC2 (130 aa; transcriptional activator protein [TrAP]), AC3 (132 aa; replication enhancer protein [REn]) and AC4 (85 aa; AC4 protein) in the complementary sense. The DNA-B components, 2603 nucleotides in length, shared more than 99.3 % nucleotide sequence identity. They had two ORFs: BV1 (256 aa; nuclear shuttle protein [NSP]) in the viral sense and BC1 (293 aa; movement protein [MP]) in the complementary sense. Both DNA components shared the conserved nonanucleotide motif (5'-TAATATTAC-3') as part of a stem-loop structure in the origin of replication (Supplementary Figure 1). The cognate DNA-A and DNA-B components had identical iterons, GGTGA, repeated two times just before the TATA-box. The corresponding iteron-related domain (IRD; Rep N-terminal domain interacting with *ori*-associated iterons [2]), was identified as MPPPKR**FRIN**.

The DNA-A and DNA-B nucleotide sequences are only distantly related to known New World bipartite begomoviruses. The DNA-A sequences share the highest nucleotide sequence identity (80 % and 79 %) with the Brazilian isolates of macroptilium yellow spot virus (MacYSV-[BR:Oaf25:11]; KC004133) and tomato chlorotic mottle virus (ToCMoV-[BR:Flo210:08]; KC706560), respectively (Fig. 1A). The DNA-B sequences share the highest nucleotide sequence identity (73 % and 72 %) with Brazilian isolates of soybean chlorotic spot virus (SBCSV-[BR:Jai9254:10]; JX122966) and tomato golden mosaic virus (TGMV-[BR:91/12], JF694491), respectively (Fig. 1B). According to the recently updated ICTV begomovirus species demarcation criteria (91 % DNA-A nucleotide sequence identity without gaps) [4]), these isolates should be assigned to a new species of tomato-infecting bipartite begomovirus, and we propose the name "tomato chlorotic mottle Guyane virus" (ToCMoGFV). ML trees confirmed that the complete nucleotide sequences of DNA-A (Fig. 1A) and DNA-B (Fig. 1B) of ToCMoGFV form a clear distinct clade among the current New World bipartite begomoviruses. Analysis of recombination using RDP4 [8] revealed no evidence of a recombination event in any of the genomic components.

In conclusion, our results show that ToCMoGFV is representative of a new lineage that is genetically isolated from the currently described New World bipartite begomoviruses in Latin America. Interestingly, the polyphagous invasive Middle East-Asia Minor 1 and Mediterranean cryptic species of *B. tabaci*, formerly known as B and Q biotypes were concomitantly detected with the disease in French Guiana (H. Delatte, unpublished results), further supporting the hypothesis that the sudden emergence of tomato-infecting begomoviruses in Latin America is mainly the result of the switch by indigenous viruses from uncultivated to cultivated host plants after the introduction and dissemination of polyphagous invasive vectors [1, 12, 13]. The natural host range of these tomato-infecting begomoviruses, the existence of undiscovered viral diversity in natural ecosystems, and the risk for crops are still open questions.

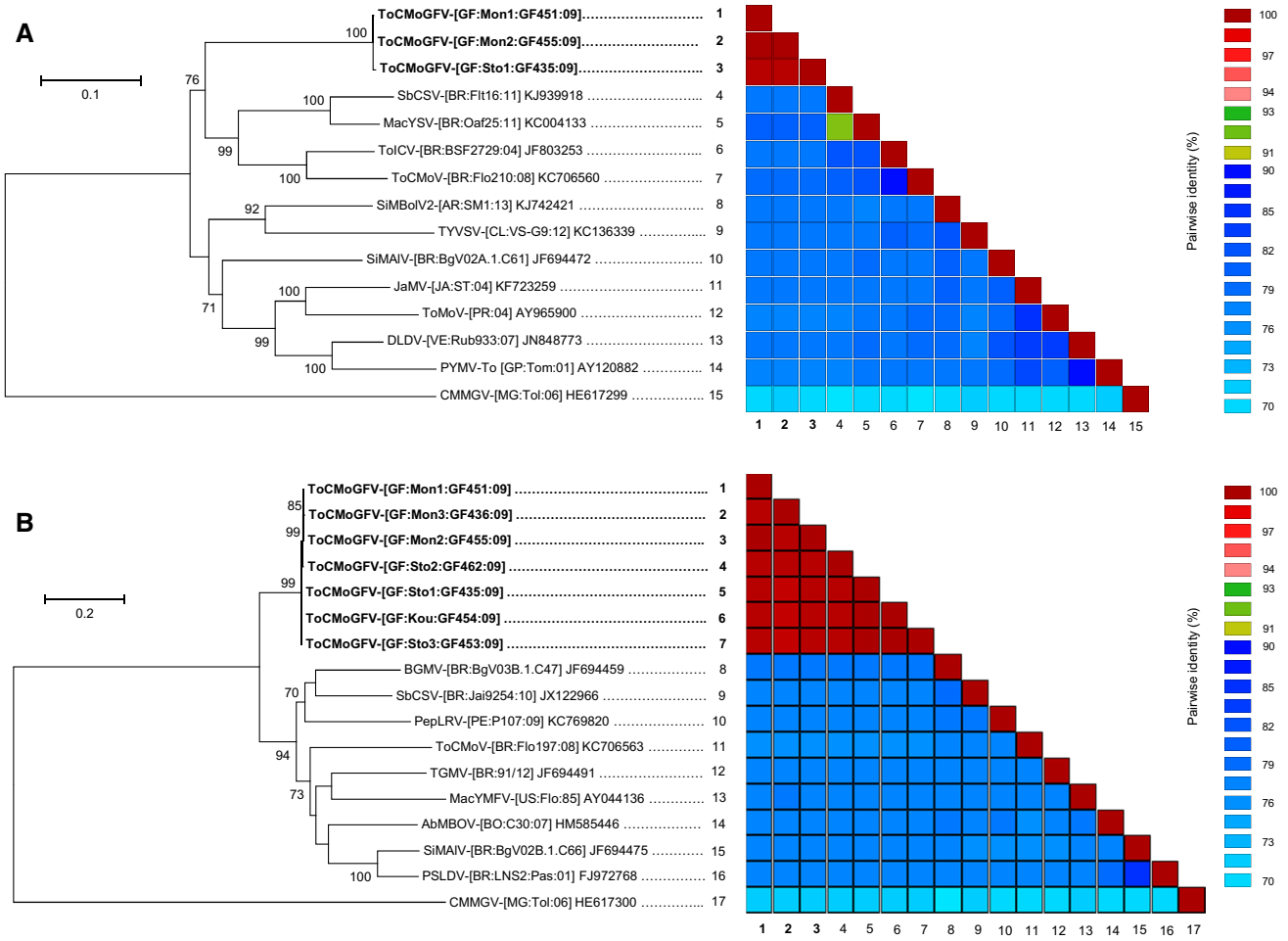

Fig. 1 Maximum-likelihood phylogenetic trees and colour-coded matrix of pairwise nucleotide sequence identity inferred from alignments of complete DNA-A (A) and DNA-B (B) sequences of ToCMoGFV and representative New World bipartite begomoviruses. Only bootstrap values at nodes higher than 70 % are displayed (1000 replicates). The scale bar represents the genetic distance. The trees were rooted with sequences of the Old World bipartite CMMGV. The matrix uses a discontinuous range of three shades of colour (red, green and blue) differentiating two cutoff values representing the strain (93-94 %, red-green) and the species (90-91 %, blue-green) demarcation thresholds of begomoviruses [4]. For begomovirus acronyms, see Supplementary Table 1 (colour figure online)

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